US ERA ARCHIVE DOCUMENT

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MRID No. 421455-01

DATA EVALUATION RECORD

- 1. CHEMICAL: Benefin. Shaughnessey No. 083401.
- 2. TEST MATERIAL: Benefin (N-(n-butyl)-N-ethyl-2,6-dinitro-«,«,«-trifluoro-p-toluidine); Lot No. 231EF4; 95.64% purity.
- 3. <u>STUDY TYPE</u>: Avian Reproduction Study. Species Tested: Mallard duck (Anas platyrhynchos).
- 4. CITATION: Murray, A.G., J.R. Smith, and D.W. Grothe. 1991.
 The Toxicity of Benefin to Mallards in a One-Generation
 Reproduction Study. Laboratory Project No. A01090. Prepared
 by Toxicology Research Laboratories, Lilly Research
 Laboratories, Greenfield, Indiana. Submitted by DowElanco,
 Indianapolis, Indiana. EPA MRID No. 421455-01.
- 5. REVIEWED BY:

Rosemary Graham Mora, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.

6. APPROVED BY:

Michael L. Whitten, M.S. Wildlife Toxicologist KBN Engineering and Applied Sciences, Inc.

Henry T. Craven, M.S. Supervisor, HED/EFED USEPA

signature:

Date

signature: Michael & White

Date: 4/23/92

Date:

- 7. CONCLUSIONS: This study is scientifically sound and fulfills the guideline requirements for an avian reproduction study. Mean measured dietary concentrations of benefin at 97 ppm, 288 ppm, and 975 ppm had no effects upon mortality, behavior, or adult food consumption in mallards during the 22-week exposure period. The NOEC was 288 ppm, due to an increase in the percentage of eggs cracked at 975 ppm.
- 8. RECOMMENDATIONS: N/A.
- 9. BACKGROUND:
- 10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- reared mallards (Anas platyrhynchos) purchased from Whistling Wings, Hanover, Illinois. All birds were of the same hatch date. For this study, 174 birds were quarantined and acclimated to the facilities for 3 weeks prior to initiation of the test. One male and one female were assigned to each pen. At test initiation, two pairs of birds were rejected due to weight loss and were replaced. The birds were 18 weeks of age at the beginning of the preproduction phase of the study.
- Dose/Diet Preparation/Food Consumption: Test diets B. were prepared on a weekly basis by dissolving the appropriate amount of benefin in acetone. This mix was added intermittently to two 8-kg batches of diet and mixed for 15 minutes. These two treated batches were combined with 104-kg batches of untreated diet and mixed for 10 minutes. The control diet and three test concentrations (100, 300, and 1,000 parts per million [ppm]) were prepared weekly and presented ad libitum to the birds. Each of the four groups of adult birds was fed the appropriate diet from test initiation until terminal sacrifice. The potency of the test material was assumed 100% for the purpose of diet preparation. Acetone accounted for no more than 1% of the total diet.

The composition of the basal diets for adult birds and their offspring was presented in the report. The test substance was not mixed into the diet of the offspring. Food and water were supplied ad libitum during acclimation and during the test for adults and offspring.

Samples of freshly prepared diets were collected on four occasions during the study for the analysis of benefin using gas chromatography. The homogeneity and stability of the test material at 100 and 1,000 ppm were determined prior to this study.

c. <u>Design</u>: The pens were assigned to treatment levels using a computer-generated table of random numbers. The test birds were distributed to the test pens using a computer-generated table of random numbers. The four groups were comprised of the following:

| Benefin Nominal | Number | Birds | Per Pen |
|--------------------|---------|-------|---------|
| Concentration | of Pens | Males | Females |
| Control (0 ppm) | 18 | 1 | 1 |
| 100 ppm | 18 | 1 | 1 |
| 300 ppm | 18 | 1 | 1 |
| 1,000 ppm | 18 | 1 | 1 |

Treatment levels were based, in part, upon acute toxicity data. Adult birds were identified by individual leg bands. The primary phases of the study and their approximate durations were as follows:

- 1. Acclimation 3 weeks
- 2. Pre-production 10 weeks
- 3. Production 12 weeks
- Post-adult sacrifice (final incubation, hatching, 14-day offspring rearing period) - 5 weeks.
- pen Facilities: Adult birds were housed indoors in pens constructed of stainless steel. Pens measured approximately 57.5 x 76.2 x 40.6 cm high. The temperature in the adult study room was approximately 21°C with an average relative humidity of 40-70%.

The photoperiod for acclimation and the preproduction period of the study was 8 hours of light per day at an intensity of 235 lux. The photoperiod was increased to 17 hours of light per day two weeks prior to the production period.

- Adult Observations/Gross Pathology: The adult and juvenile birds were observed twice daily on the weekdays and once daily on the weekends and holidays throughout the study for signs of toxicity or abnormal behavior. All birds that died during the study were necropsied. The pen mate of dead birds was sacrificed and necropsied. At study termination, all surviving birds were sacrificed and necropsied. Adult birds were weighed at test initiation, weekly during acclimation and the preproduction period, and at study termination. Food consumption per pen was determined weekly throughout the study.
- F. Eggs/Eggshell Thickness: Eggs were collected daily, marked (date, pen number, and treatment level), candled, and stored in a refrigerator at 15°C. Cracked eggs or soft-shelled eggs were recorded and discarded. All cracked eggs for Set 4 and 6 were measured for

eggshell thickness and discarded. The remaining eggs were incubated in an incubator with a dry bulb temperature of 100°F and a wet bulb temperature of 85-90°F. The eggs were candled on days 14 and 21. On day 24 of incubation, live eggs were transferred to a hatcher with a dry bulb temperature of 98-100°F and a wet bulb temperature of 85-100°F.

When possible, the 7th, 14th, and 21st egg from each pen was collected for eggshell thickness determination. These eggs were opened at the girth, the contents removed, and the shell washed thoroughly to remove the albumen and allowed to air dry at room temperature for at least 48 hours. The average thickness of the dried shell plus membrane was determined by measuring (to the nearest 0.001 mm) two points around each half of the waist of the egg using a micrometer.

- Ratchlings: All hatchlings and unhatched eggs were removed from the hatcher on day 27 of incubation. The average body weight of the hatchlings by pen was then determined. Hatchlings were wingbanded for identification by parental pen and placed in brooding pens until 14 days of age. Each brooding pen measured 43.2 x 75.2 x 27.9 cm high with plastic-coated wire mesh floors. Each brooder was equipped with two heat lamps which maintained the temperature in the brooding pens at approximately 37°C during the 14-day survival period. Continuous light was provided. Relative humidity was maintained at 25-60%. At 14 days of age, the average body weight of all survivors was determined.
- statistics: Upon completion of the study, two-factor H. repeated measures analysis of variance (ANOVA) was used to assess the effects of benefin on adult body weight, hatchling body weight, and hatchling body weight gain. All other variables were analyzed using a one-way Proportional data (e.g., EC/EL and VE/ES) were subjected to arcsine transformation prior to analysis. "F-statistics were used to test the statistical significance of all main effect and interactions terms. Additionally, the statistical significance of linear trends across the concentration levels of benefin were examined to determine the concentration level below which no significant trend could be detected. references to statistical significance represent ". 20.05 g

Each of the following parameters was analyzed statistically:

Offspring Body Weight Adult Body Weight Offspring Food Adult Feed Consumption Consumption Egg Production 14-Day Old Survivors Eggs Laid per Hen Number of Eggs Laid 14-Day Survivors Eggs Cracked of Eggs Laid per Hen 14-Day Old Survivors of Viable Embryos of Eggs Set Live 3-Week Embryos of Eggs Laid 14-Day Old Survivors of Viable Embryos of Hatchlings Hatchlings of 3-Week Egg Shell Thickness Embryos

12. REPORTED RESULTS

<u>Diet Analysis</u>: Analyses conducted prior to this study showed that homogeneity and stability after 2 weeks were within acceptable limits. The results of the diet analyses are presented in Table 2 (attached). Nominal and mean measured concentrations of freshly prepared diets were as follows:

| Benefin | (mqq) | |
|--------------------------|--------------------------------|-----------------------|
| Nominal Concentration | Mean Measured Concentration | Percent of Nominal |
| 0 | <2 | NA |
| 100 | 97 | 97% |
| 300 | 288 | 96% |
| 1.000 | 975 | 97.5% |

Subsequent discussions refer to the treatment groups using their mean measured concentrations.

B. <u>Mortality and Behavioral Reactions</u>: "No signs of toxicity were observed at any treatment level." One incidental mortality occurred in the control, 97-ppm group, and 288-ppm group.

Necropsy results of all mortalities and sacrificed birds were included in the report. "No compoundrelated gross or microscopic lesions were detected."

c. <u>Adult Body Weight and Food Consumption</u>: There were no significant differences in food consumption during the preproduction period between the control and treatment levels (Table 8, attached). A significant decrease in food consumption during the production period was noted

at the two lower test concentrations (Table 9, attached). However, no significant decrease was noted at the higher test level. Therefore, the slight decrease noted at the two lower test levels "does not appear to be related to increasing concentrations of benefin."

There were no apparent treatment related effects upon body weight or weight gain among birds at any of the concentrations tested (Tables 4-7, attached).

- P. Reproduction: When compared to the control group, there were no statistically significant differences in reproductive parameters, except the percentage of eggs cracked/eggs laid, at any concentration tested (Tables 10-12, attached). The ratio of eggs cracked/eggs laid was significantly higher at the highest test level (975 ppm) when compared to the control.
- E. Egg Shell Thickness: Eggshell thickness for cracked eggs and normal eggs was not affected by treatment. There was no significant difference between the control and test concentrations in mean egg shell thickness for normal eggs. Cracked control eggs were significantly thinner than normal eggs and thinner than cracked eggs from all treatment levels (Table 13, attached).
- f. Offspring Body Weight: When compared to the control, there was no significant reduction in mean body weights or body weight gain in any treatment group (Tables 16 and 17, attached). "Mean food consumption was slightly lower for hatchlings fed benefin when compared to the control." Mean offspring food consumption (g/bird/day) was as follows: 81 (control), 75 (97 ppm), 77 (288 ppm), and 67 (975 ppm).
- 13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:
 "Based upon the results of this study and maximum use rate patterns, benefin is not expected to have any adverse effect to mallards." The NOEC was 288 ppm, due to an increase in the percentage of cracked eggs at 975 ppm.

The report stated that the study was conducted in compliance with EPA (FIFRA 40 CFR, Part 160), OECD and Japanese MAFF GLP standards. Quality assurance audits were conducted during the study and the final report was signed by the Quality Assurance Representative and the Study Director.

14. Reviewer's Discussion and Interpretation of the Study:

A. <u>Test Procedure</u>: The test procedures were in accordance with Subdivision E - Hazard Evaluation: Wildlife and Aquatic Organisms, ASTM, and SEP guidelines except for the following deviations:

The physical properties of the test material (i.e., powder, liquid) were not reported.

A withdrawal study period (using basal diet only) was not added to the test phase.

For this study, the light intensity provided to the test birds was 235 lux (22 footcandles). The light intensity recommended by the guidelines is 65 lux (6 footcandles).

During the preproduction phase of this study, the period of light provided to adult birds was 8 hours. The SEP recommends 7 hours of light.

For this study, the temperature in the offspring pens was 37°C. ASTM guidelines recommend a temperature gradient from the heat source to about 21°C in order to allow the birds to seek a proper temperature.

The report did not indicate whether the mallards used in this study were phenotypically indistinguishable from wild mallards as recommended.

Behavioral observations of offspring were not reported.

Observations on food palatability were not reported.

All eggs were transferred to the hatcher on day 24. The guidelines recommend the transfer on day 23.

On page 29 of the report, the authors state, "Mean food consumption was slightly lower for hatchlings fed benefin when compared to the control." However, benefin was not added to the hatchling diet (TEKLAD AN11DU) (pages 16 and 18). This is assumed to be a discrepancy in the report, rather than a deviation in procedures.

B. <u>Statistical Analysis</u>: Statistical analyses of study parameters were performed by the reviewer using analysis of variance (ANOVA) following square-root transformation of the count data and arcsine square-root transformation of the ratio data. The comparison between control data and data from each treatment level

was made using multiple comparison tests. The computer program used is based on the EEB Bigbird program, with an exception that the count data were square-root transformed before the ANOVA. The significance level was $p \leq 0.05$.

Analyses of study parameters were verified (attached) and found to match those reported by the author, with the exception of the percentage of eggs cracked. The authors reported a significant difference between the ratio of eggs cracked/eggs laid in the control and those of the 975 ppm group (p=0.03), while the reviewer's analysis showed no significant difference. However, the reviewer's analysis showed a difference at 975 ppm approaching the level of significance (p=0.089 for eggs cracked and p=0.061 for eggs laid/eggs cracked). Therefore, a conservative approach would be to assume that this is a treatment-related effect.

The reduced food consumption at 97 and 288 ppm does not appear to be a treatment effect.

c. <u>Discussion/Results</u>: This study is scientifically sound and fulfills the guideline requirements for an avian reproduction study. The mean measured dietary concentrations of benefin (97 ppm, 288 ppm, and 975 ppm) had no effects upon mortality, behavior, adult food consumption, or adult body weights in mallards during the 22-week exposure period. However, due to a high percentage of cracked eggs at 975 ppm, the NOEC was 288 ppm.

D. Adequacy of the Study:

- (1) Classification: Core.
- (2) Rationale: Deviations from protocols were minor and did not affect the validity of the study.
- (3) Repairability: N/A.
- 15. COMPLETION OF ONE-LINER: Yes; April 7, 1992.

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| CASE | 2 | U | 39 | 1 | 35 | 28 |
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| CASE | 5 | | 46 | 3 | 40 | 34 |
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| CASE | 8 | | 45 | 2 | 40 | 37 |
| CASE | 9 | | 61 | 8 | 48 | 41 |
| CASE | 10 | | 61 | 2 | 56 | 51 |
| CASE | 11 | | 56 | 6 | 47 | 43 |
| CASE | 12 | | 69 | 17 | 49 | 44 |
| CASE | 13 | | 36 | 2 | 31 | 30 |
| CASE | 14 | | 57 | 1 | 53 | 52 |
| CASE | 15 | | 31 | 0 | 28 | 27 |
| CASE | 16 | \'/ | 62 | 1 | 58 | 55 |
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| CASE | 18 | | 54 | 5 | 46 | 45 |
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| CASE | 20 | 1 | 33 | 0 | 30 | 29 |
| CASE | 21 | | 57 | 4 | 50 | 48 |
| CASE | 22 | - 1 | 60 | 1 | 56 | 49 |
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| CASE | 24 | | 63 | 0 | 60 | 55 |
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BENEFIN: MALLARD REPRODUCTION

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ANOVA on SQR(Eggs Laid)

| | LEVELS | ENCOUNTERED | DURING | PROCESSING | ARE: |
|--|---------------|-------------|--------|------------|------|
|--|---------------|-------------|--------|------------|------|

TRT

0.000

1.000

2.000

3.000

71 MULTIPLE R: 0.251 SQUARED MULTIPLE R: 0.063 DEP VAR: SEL N:

ANALYSIS OF VARIANCE

| SOURCE | SUM-OF-SQUARES | DF | MEAN-SQUARE | F-RATIO | P |
|--------|----------------|----|-------------|---------|-------|
| TRT | 11.110 | 3 | 3.703 | 1.506 | 0.221 |
| ERROR | 164.793 | 67 | 2.460 | | |

Post-hoc contrast of treatment 1 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | .F | P |
|---------------------|------------------|---------|----------------|-----------|-------|
| HYPOTHESIS ERROR | 9.415 164.793 | 1 67 | 9.415 2.460 | 3.828 | 0.055 |

Post-hoc contrast of treatment 2 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|------------------|---------|----------------|-------|-------|
| HYPOTHESIS ERROR | 0.597 164.793 | 1 67 | 0.597 2.460 | 0.243 | 0.624 |

Post-hoc contrast of treatment 3 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|------------|------------------|---------|----------------|-------|-------|
| HYPOTHESIS | 0.237 164.793 | 1 67 | 0.237 2.460 | 0.096 | 0.757 |

ANOVA on SQR(Eggs Cracked)

LEVELS ENCOUNTERED DURING PROCESSING ARE:

TRT

0.000 1.000 2.000 3.000

ANALYSIS OF VARIANCE

MEAN-SQUARE F-RATIO P SUM-OF-SQUARES DF SOURCE 1.709 0.174 2.510 3 TRT 7.530 66 1.469 ERROR 96.942

Post-hoc contrast of treatment 1 with control.

TEST FOR EFFECT CALLED:

TRT

TEST OF HYPOTHESIS

Р F MS SOURCE SS DF 0.675 0.260 0.177 0.260 1 HYPOTHESIS 96.942 66 1.469 ERROR

Post-hoc contrast of treatment 2 with control.

TEST FOR EFFECT CALLED:

 $\mathbf{T}\mathbf{R}\mathbf{T}$

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|-----------------|---------|----------------|-------|-------|
| HYPOTHESIS ERROR | 0.336 96.942 | 1 66 | 0.336 1.469 | 0.229 | 0.634 |

Post-hoc contrast of treatment 3 with control.

TEST FOR EFFECT CALLED:

TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|-----------------|---------|----------------|-------|-------|
| HYPOTHESIS ERROR | 4.386 96.942 | 1 66 | 4.386 1.469 | 2.986 | 0.089 |

ANOVA on SQR(Eggs Set)

LEVELS ENCOUNTERED DURING PROCESSING ARE:

TRT

0.000

1.000

2.000

3.000

ANALYSIS OF VARIANCE

| SOURCE | SUM-OF-SQUARES | DF | MEAN-SQUARE | F-RATIO | P . |
|--------|----------------|----|-------------|---------|------------|
| TRT | 4.591 | .3 | 1.530 | 0.643 | 0.590 |
| ERROR | 157.041 | 66 | 2.379 | | |

Post-hoc contrast of treatment 1 with control.

TEST FOR EFFECT CALLED:

TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | ŕ | P |
|---------------------|------------------|---------|----------------|-------|-------|
| HYPOTHESIS ERROR | 4.465 157.041 | 1 66 | 4.465 2.379 | 1.877 | 0.175 |

Post-hoc contrast of treatment 2 with control.

TEST FOR EFFECT CALLED:

 $\mathbf{T}\mathbf{R}\mathbf{T}$

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|------------------|---------|----------------|-------|-------|
| HYPOTHESIS ERROR | 1.745 157.041 | 1 66 | 1.745 2.379 | 0.733 | 0.395 |

Post-hoc contrast of treatment 3 with control.

TEST FOR EFFECT CALLED:

TRT

TEST OF HYPOTHESIS

| SOURCE | ss | DF | MS | F | P |
|---------------------|------------------|---------|----------------|-------|-------|
| HYPOTHESIS ERROR | 1.511 157.041 | 1 66 | 1.511 2.379 | 0.635 | 0.428 |

ANOVA on SQR(Viable Embryos)

LEVELS ENCOUNTERED DURING PROCESSING ARE:

TRT

0.000

1.000

2.000

3.000

ANALYSIS OF VARIANCE

SOURCE SUM-OF-SQUARES DF MEAN-SQUARE F-RATIO P

TRT 6.468 3 2.156 0.772 0.514

ERROR 184.316 66 2.793

Post-hoc contrast of treatment 1 with control.

TEST FOR EFFECT CALLED:

TRT

TEST OF HYPOTHESIS

F P MS SOURCE SS DF 1.382 0.244 3.859 HYPOTHESIS 3.859 1 184.316 66 2.793 ERROR

Post-hoc contrast of treatment 2 with control.

TEST FOR EFFECT CALLED:

TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|------------------|---------|----------------|-------|-------|
| HYPOTHESIS ERROR | 5.418 184.316 | 1 66 | 5.418 2.793 | 1.940 | 0.168 |

Post-hoc contrast of treatment 3 with control.

TEST FOR EFFECT CALLED:

TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | \mathbf{P} |
|---------------------|------------------|---------|----------------|-------|--------------|
| HYPOTHESIS ERROR | 1.246 184.316 | 1 66 | 1.246 2.793 | 0.446 | 0.506 |

ANOVA on SQR(21-day Live Embryos)

LEVELS ENCOUNTERED DURING PROCESSING ARE:

TRT

0.000 1.000

2.000

3.000

ANALYSIS OF VARIANCE

| SOURCE | SUM-OF-SQUARES | DF | MEAN-SQUARE | F-RATIO | P |
|--------|----------------|----|-------------|---------|-------|
| TRT | 6.419 | 3 | 2.140 | 0.766 | 0.517 |
| ERROR | 184.364 | 66 | 2.793 | | |

Post-hoc contrast of treatment 1 with control.

TEST FOR EFFECT CALLED:

TEST OF HYPOTHESIS

P F MS DF SOURCE SS 0.229 1.471 1 4.110 HYPOTHESIS 4.110 2.793 184.364 66 ERROR

Post-hoc contrast of treatment 2 with control.

TEST FOR EFFECT CALLED:

TRT

TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|------------------|---------|----------------|-------|-------|
| HYPOTHESIS ERROR | 5.131 184.364 | 1 66 | 5.131 2.793 | 1.837 | 0.180 |

Post-hoc contrast of treatment 3 with control.

TEST FOR EFFECT CALLED:

TRT

TEST OF HYPOTHESIS

| SOURCE | ss | DF | MS | F | P |
|---------------------|------------------|---------|----------------|-------|-------|
| HYPOTHESIS ERROR | 1.195 184.364 | 1 66 | 1.195 2.793 | 0.428 | 0.515 |

ANOVA on SQR(Hatched)

LEVELS ENCOUNTERED DURING PROCESSING ARE:

TRT

1.000

2.000

3.000

SHAT N: 70 MULTIPLE R: 0.086 SQUARED MULTIPLE R: 0.007 DEP VAR:

ANALYSIS OF VARIANCE

SOURCE SUM-OF-SQUARES DF MEAN-SQUARE F-RATIO P

TRT 1.427 3 0.476 0.164 0.920

2.900 ERROR 191.417 66

Post-hoc contrast of treatment 1 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

DF F P SOURCE MS SS 0.236 0.629 HYPOTHESIS 0.683 1 0.683 191.417 2,900 ERROR 66

Post-hoc contrast of treatment 2 with control.

TEST FOR EFFECT CALLED:

TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|------------------|---------|----------------|-------|-------|
| HYPOTHESIS ERROR | 1.335 191.417 | 1 66 | 1.335 2.900 | 0.460 | 0.500 |

Post-hoc contrast of treatment 3 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|------------------|---------|----------------|-------|-------|
| HYPOTHESIS ERROR | 0.417 191.417 | 1 66 | 0.417 2.900 | 0.144 | 0.706 |

ANOVA on SQR(Two week Survivors)

LEVELS ENCOUNTERED DURING PROCESSING ARE:

TRT

0.000

1.000

2.000

3.000

ANALYSIS OF VARIANCE

SUM-OF-SQUARES DF MEAN-SQUARE F-RATIO P SOURCE 0.811 0.831 0.320 3 2.492 TRT 2.599 171.549 66 ERROR

Post-hoc contrast of treatment 1 with control.

TEST FOR EFFECT CALLED:

TRT

TEST OF HYPOTHESIS

P MS F DF SOURCE SS 0.645 0.557 0.214 HYPOTHESIS 0.557 1 2.599 171.549 66 ERROR

Post-hoc contrast of treatment 2 with control.

TEST FOR EFFECT CALLED:

TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | , P |
|---------------------|------------------|---------|----------------|-------|------------|
| HYPOTHESIS ERROR | 2.234 171.549 | 1 66 | 2.234 2.599 | 0.860 | 0.357 |

Post-hoc contrast of treatment 3 with control.

TEST FOR EFFECT CALLED:

TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|------------------|---------|----------------|-------|-------|
| HYPOTHESIS ERROR | 0.107 171.549 | 1 66 | 0.107 2.599 | 0.041 | 0.840 |

ANOVA on EC/EL

LEVELS ENCOUNTERED DURING PROCESSING ARE:

TRT

0.000 1.000 2.000

3.000

DEP VAR: RESP1 N: 70 MULTIPLE R: 0.306 SQUARED MULTIPLE R: 0.094

ANALYSIS OF VARIANCE

| SOURCE | SUM-OF-SQUARES | DF | MEAN-SQUARE | F-RATIO | P |
|--------|----------------|----|-------------|---------|-------|
| TRT | 804.830 | 3 | 268.277 | 2.274 | 0.088 |
| ERROR | 7785.896 | 66 | 117.968 | | |

Post-hoc contrast of treatment 1 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|--------------------|---------|-------------------|-------|-------|
| HYPOTHESIS ERROR | 24.620 7785.896 | 1 66 | 24.620 117.968 | 0.209 | 0.649 |

Post-hoc contrast of treatment 2 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|---------------------|---------|--------------------|-------|-------|
| HYPOTHESIS ERROR | 151.506 7785.896 | 1 66 | 151.506 117.968 | 1.284 | 0.261 |

Post-hoc contrast of treatment 3 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | ss | DF | MS | F | P |
|---------------------|---------------------|---------|--------------------|-------|-------|
| HYPOTHESIS ERROR | 429.353 7785.896 | 1 66 | 429.353 117.968 | 3.640 | 0.061 |

ANOVA on VE/ES

| LEVELS | ENCOUNTERED | DURING | PROCESSING | ARE: |
|--------|-------------|--------|------------|------|
|--------|-------------|--------|------------|------|

TRT

1.000 2.000 0.000

3.000

69 MULTIPLE R: 0.038 SQUARED MULTIPLE R: 0.001 N: RESP2 DEP VAR:

ANALYSIS OF VARIANCE

F-RATIO P SUM-OF-SQUARES DF MEAN-SQUARE SOURCE 0.992 0.031 TRT 16.736 3 5.579 11564.154 65 177.910 ERROR

Post-hoc contrast of treatment 1 with control.

TEST FOR EFFECT CALLED:

TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|--------------------|---------|------------------|-------|-------|
| HYPOTHESIS ERROR | 0.199 11564.154 | 1 65 | 0.199 177.910 | 0.001 | 0.973 |

Post-hoc contrast of treatment 2 with control.

TEST FOR EFFECT CALLED:

TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|--------------------|---------|------------------|-------|-------|
| HYPOTHESIS ERROR | 6.378 11564.154 | 1 65 | 6.378 177.910 | 0.036 | 0.850 |

Post-hoc contrast of treatment 3 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|---------------------|---------|-------------------|-------|-------|
| HYPOTHESIS ERROR | 12.587 11564.154 | 1 65 | 12.587 177.910 | 0.071 | 0.791 |

ANOVA on LE21/VE

LEVELS ENCOUNTERED DURING PROCESSING ARE:

TRT

0.000

1.000

2.000

3.000

DEP VAR: RESP3 N: 68 MULTIPLE R: 0.083 SQUARED MULTIPLE R: 0.007

ANALYSIS OF VARIANCE

SUM-OF-SQUARES DF MEAN-SQUARE F-RATIO SOURCE 7.513 0.147 0.931 22.540 3 TRT 3263.906 64 50.999 ERROR

Post-hoc contrast of treatment 1 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|-------------------|---------|-----------------|-------|-------|
| HYPOTHESIS ERROR | 4.203 3263.906 | 1 64 | 4.203 50.999 | 0.082 | 0.775 |

Post-hoc contrast of treatment 2 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|--------------------|---------|------------------|-------|-------|
| HYPOTHESIS ERROR | 18.453 3263.906 | 1 64 | 18.453 50.999 | 0.362 | 0.550 |

Post-hoc contrast of treatment 3 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|--------------------|---------|------------------|-------|-------|
| HYPOTHESIS ERROR | 14.137 3263.906 | 1 64 | 14.137 50.999 | 0.277 | 0.600 |

ANOVA on HAT/LE21

LEVELS ENCOUNTERED DURING PROCESSING ARE:

TRT

0.000 1.000 2.000 3.000

68 MULTIPLE R: 0.195 SQUARED MULTIPLE R: 0.038 DEP VAR: RESP4 N:

ANALYSIS OF VARIANCE

SUM-OF-SQUARES DF MEAN-SQUARE F-RATIO SOURCE 0.473 0.848 178.450 3 TRT 535.351

64 210.499 13471.965 ERROR

Post-hoc contrast of treatment 1 with control.

TEST FOR EFFECT CALLED:

TEST OF HYPOTHESIS

SS DF MS F SOURCE 2.093 0.153 HYPOTHESIS 440.570 1 440.570 64 210.499 13471.965 ERROR

Post-hoc contrast of treatment 2 with control.

TEST FOR EFFECT CALLED:

TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|---------------------|---------|-------------------|-------|-------|
| HYPOTHESIS ERROR | 28.538 13471.965 | 1 64 | 28.538 210.499 | 0.136 | 0.714 |

Post-hoc contrast of treatment 3 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | ss | DF | MS | F | P |
|---------------------|--------------------|---------|------------------|-------|-------|
| HYPOTHESIS ERROR | 3.488 13471.965 | 1 64 | 3.488 210.499 | 0.017 | 0.898 |

ANOVA on TWOWK/HAT

LEVELS ENCOUNTERED DURING PROCESSING ARE:

TRT

3.000 2.000 1.000

DEP VAR: RESP5 N: 68 MULTIPLE R: 0.202 SQUARED MULTIPLE R: 0.041

ANALYSIS OF VARIANCE

SUM-OF-SQUARES DF MEAN-SQUARE F-RATIO P SOURCE

0.905 0.444 3 191.196 TRT 573.589

13527.810 64 211.372 ERROR

Post-hoc contrast of treatment 1 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

SS DF MS F SOURCE 92.384 0.511 1 92.384 0.437 HYPOTHESIS 13527.810 64 211.372 ERROR

Post-hoc contrast of treatment 2 with control.

TEST FOR EFFECT CALLED:

TRT

TEST OF HYPOTHESIS

SS DF MS F P SOURCE 0.056 0.815 11.732 1 HYPOTHESIS 11.732 211.372 64 ERROR 13527.810

Post-hoc contrast of treatment 3 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|----------------------|---------|--------------------|-------|-------|
| HYPOTHESIS ERROR | 343.518 13527.810 | 1 64 | 343.518 211.372 | 1.625 | 0.207 |

ANOVA on HAT/ES

LEVELS ENCOUNTERED DURING PROCESSING ARE:

1.000 0.000

2.000

3.000

69 MULTIPLE R: 0.148 SQUARED MULTIPLE R: 0.022 DEP VAR: RESP6 N:

ANALYSIS OF VARIANCE

| SOURCE | SUM-OF-SQUARES | DF | MEAN-SQUARE | F-RATIO | P |
|--------|----------------|----|-------------|---------|-------|
| TRT | 306.671 | 3 | 102.224 | 0.484 | 0.694 |
| ERROR | 13715.914 | 65 | 211.014 | | |

Post-hoc contrast of treatment 1 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|----------------------|---------|--------------------|-------|-------|
| HYPOTHESIS ERROR | 188.921 13715.914 | 1 65 | 188.921 211.014 | 0.895 | 0.348 |

Post-hoc contrast of treatment 2 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|--------------------|---------|------------------|-------|-------|
| HYPOTHESIS ERROR | 8.688 13715.914 | 1 65 | 8.688 211.014 | 0.041 | 0.840 |

Post-hoc contrast of treatment 3 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | ss | DF | MS | F | P |
|---------------------|--------------------|---------|------------------|-------|-------|
| HYPOTHESIS ERROR | 9.009 13715.914 | 1 65 | 9.009 211.014 | 0.043 | 0.837 |

ANOVA on TWOWK/ES

LEVELS ENCOUNTERED DURING PROCESSING ARE:

TRT

0.000

1.000

2.000

3.000

DEP VAR: RESP7 N: 69 MULTIPLE R: 0.175 SQUARED MULTIPLE R: 0.031

ANALYSIS OF VARIANCE

| SOURCE | SUM-OF-SQUARES | DF | MEAN-SQUARE | F-RATIO | P |
|--------|----------------|----|-------------|---------|-------|
| TRT | 379.139 | 3 | 126.380 | 0.688 | 0.563 |
| ERROR | 11938.949 | 65 | 183.676 | | |

Post-hoc contrast of treatment 1 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|------------|-----------|----|---------|-------|-------|
| HYPOTHESIS | 114.549 | 1 | 114.549 | 0.624 | 0.433 |
| ERROR | 11938.949 | 65 | 183.676 | | |

Post-hoc contrast of treatment 2 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|---------------------|---------------------|---------|-------------------|-------|-------|
| HYPOTHESIS ERROR | 68.375 11938.949 | 1 65 | 68.375 183.676 | 0.372 | 0.544 |

Post-hoc contrast of treatment 3 with control.

TEST FOR EFFECT CALLED: TRT

TEST OF HYPOTHESIS

| SOURCE | SS | DF | MS | F | P |
|------------|-----------|----|---------|-------|-------|
| HYPOTHESIS | 28.519 | 1 | 28.519 | 0.155 | 0.695 |
| ERROR | 11938.949 | 65 | 183.676 | | |

SUMMARY STATISTICS FOR EL

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

CHI-SOUARE = 11.060 DF= 3 PROBABILITY = 0.011

ANALYSIS OF VARIANCE

SUM OF SQUARES DF MEAN SQUARE F PROBABILITY SOURCE

262.644 0.888 0.452 BETWEEN GROUPS 787.932 3

295.687 19811.026 67 WITHIN GROUPS

SUMMARY STATISTICS FOR EC

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

4.701 DF= 3 PROBABILITY = 0.195 CHI-SOUARE =

ANALYSIS OF VARIANCE

SUM OF SQUARES DF MEAN SQUARE F PROBABILITY SOURCE

74.232 3 2072.111 66 24.744 0.788 0.505 BETWEEN GROUPS WITHIN GROUPS

31.396

SUMMARY STATISTICS FOR ES

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

CHI-SQUARE = 10.774 DF= 3 PROBABILITY = 0.013

ANALYSIS OF VARIANCE

SOURCE SUM OF SQUARES DF MEAN SQUARE F PROBABILITY

337.672 3 112.557 0.416 0.742 BETWEEN GROUPS
WITHIN GROUPS 17854.971 66 270.530

SUMMARY STATISTICS FOR VE

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

CHI-SQUARE = 14.299 DF= 3 PROBABILITY = 0.003

ANALYSIS OF VARIANCE

SOURCE SUM OF SQUARES DF MEAN SQUARE F PROBABILITY

266.936 3 17259.007 66 0.340 0.796 BETWEEN GROUPS
WITHIN GROUPS 88.979

261.500

SUMMARY STATISTICS FOR LE21

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

CHI-SQUARE = 14.591 DF= 3 PROBABILITY = 0.002

ANALYSIS OF VARIANCE

SOURCE SUM OF SQUARES DF MEAN SQUARE F PROBABILITY

BETWEEN GROUPS 261.636 3 87.212 0.338 0.798

WITHIN GROUPS 17009.850 66 257.725

SUMMARY STATISTICS FOR HAT

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

CHI-SQUARE = 2.964 DF= 3 PROBABILITY = 0.397

ANALYSIS OF VARIANCE

SOURCE SUM OF SQUARES DF MEAN SQUARE F PROBABILITY

BETWEEN GROUPS 32.733 3 10.911 0.058 0.982

WITHIN GROUPS 12502.353 66 189.430

SUMMARY STATISTICS FOR TWOWK

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

CHI-SQUARE = 2.850 DF= 3 PROBABILITY = 0.415

ANALYSIS OF VARIANCE

SOURCE SUM OF SQUARES DF MEAN SQUARE F PROBABILITY

BETWEEN GROUPS 40.411 3 13.470 0.085 0.968

WITHIN GROUPS 10449.889 66 158.332

BENEFIN: MALLARD REPRODUCTION

KOLMOGOROV-SMIRNOV ONE SAMPLE TEST USING STANDARD NORMAL DISTRIBUTION

| VARIABLE | N-OF-CASES | MAXDIF PROBA | BILITY (2-TAIL) |
|----------|------------|--------------|-----------------|
| EL | 71.000 | 0.972 | 0.000 |
| EC | 70.000 | 0.670 | 0.000 |
| ES | 70.000 | 0.986 | 0.000 |
| VE | 70.000 | 0.971 | 0.000 |
| LE21 | 70.000 | 0.971 | 0.000 |
| ТАН | 70.000 | 0.934 | 0.000 |
| TWOWK | 70.000 | 0.934 | 0.000 |
| | | | |